CLAIMS

1. A process for the production of egg yolk antibodies binding to small molecule organo chlorine pesticides, the said process comprising the steps of:
   (a) selecting suitable from poultry birds;
   (b) immunizing the poultry birds with known complete adjuvant, each ml of said adjuvant comprising heat killed and dried 1 mg of Mycobacterium tuberculosis (H37Ra, ATCC 25177), 0.85 ml paraffin and 0.15 ml mannide monooleate;
   (c) immunizing the birds with 1000 µg conjugate selected from DDT-OH hapten, Octachloro cyclic hapten, 2,4,5 trichlorophoxyacetic acid β-alanine in breast muscle;
   (d) immunizing the birds again with the hapten-protein conjugate as given in step (c) with 500 µg of desired hapten conjugate;
   (e) immunizing the birds with hapten-protein conjugate at the intervals of two, three and five weeks;
   (f) immunizing the birds thereafter with hapten-protein conjugate at five weeks intervals as long as the bird lays eggs;
   (g) harvesting antibodies from the egg yolk of the birds.

2. A process as claimed in claim 1, wherein the desired hapten-protein conjugates having binding properties to DDT, Endosulphan and Hexachlorohexane .

3. A process as claimed in claim 1, wherein the production of hapten-protein conjugate, namely DDT-OH binding to DDT, as used in step (c) of claim 1, is as follows:
   (a) succinylating 2,2,-Bis(4-chlorophenyl) –1,1,1-trichloroethanol overnight, using excess succinic anhydride in pyridine to obtain N-hydroxy succunimide;
   (b) reacting N-hydroxy succinimide 183.5 mg., 0.5mmol in dichloromethane in the presence of dicyclohexylurea and dimethylaminopyridine catalyst in the ratio 1:1:1:1.2 (hapten:NHS:DCC:DMAP) to convert into N-Hydroxy succinimide active ester; and

(c) obtaining active ester of DDT-OH hapten for use in conjugation by isolating dicyclohexylurea and evaporating dichloromethane.

4. A process as claimed in claim1, wherein the production of hapten-protein conjugate namely octachloro cyclic hapten binding to Endosulphan as used in step(c) of claim 1, is as follows:
   (a) dissolving about 3.73 g Heptachlor in 0.1 mol glacial acetic acid by warming;
   (b) dissolving 1.085 g Tert-Butyl hypochlorite, in 0.1 mmol glacial acetic acid and adding to the first solution as obtained in step(a);
   (c) refluxing the mixture on a water-bath for 1 hour;
   (d) separating fine crystals of acetyl-chloro derivative of heptachlor;
   (e) washing the crystals with acetone and drying with air;
   (f) obtaining the crystalline product in a yield of about 3.02 g, m.p. 238 C. 1.09 g
   (g) treating the product to get the pre-hapten 1,3,4,5,6,7,8,8-Octachloro-2-hydroxy-4, 7-methano-3a, and 4,7,7a-tetrahydroindane;
   (h) dissolving the pre hapten in dichloromethane by adding N-hydroxysuccinimide and cooling the mixture to 0^0C ;
   (i) adding dicyclohexylcarbodiimide followed by dimethylaminopyridine;
   (j) stirring the mixture overnight; and
   (k) filtering off dichloromethane and evaporating dichloromethane to obtain the active ester of endosulphan.

5. A process as claimed in claim 1, wherein the production of conjugate hapten 2,4,5-Trichloro phenoxy acetic acid β- alanine (TCB) hapten binding to Hexachloro hexane, as per step(c), is as follows:
   (a) adding of ß – alanine spacer arm to 2,4,5 Trichlorophenoxyacetic acid by suspending 10mM, 2.55g of 2,4,5 Trichlorophenoxyacetic acid in 5.95 ml thionyl chloride (9 50 mmol);
   (b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation;

(c) stirring the product with ß – alanine (9 mmol, 0.66g in 7.4 ml of 1M NaOH) at 0^0C;

(d) warming the product for over 16 hours at room temperature;

(e) isolating the resulting acid by acidification;

(f) partitioning into ethyl acetate;

(g) washing with water and brine;

(h) giving an yield of crude product hapten containing 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) as impurity;

(i) dissolving the impurity in acetone to obtain colorless flakes of the Trichlorobenzene(TCB) hapten;

(j) filtering and washing the colourless TCB hapten with acetone and drying in air;

(k) using silica gel precoated aluminum plates and a mixture of chloroform and methanol in a ratio of 85:15 as eluent showed a single spot in TLC analysis Rf-0.45 detected by spraying with 2% o-tolidine in acetone and exposure to Uv light/sunlight, at a melting range of 169-70 ° C.

(l) synthesizing the active ester of hapten 2,4,5-T-β- alanine at melting range of 102-104 °C by dissolving in dichloromethane .

(m) adding N- hydroxysuccinimide and the mixture is cooled in an ice-bath;

(n) adding Dimethylsulphoxide(DMSO) dropwise to the mixture until the hapten is dissolved;

(o) adding Dicyclohexylcarbodiimide to the mixture followed by adding dimethylaminopyridine catalyst;

(p) stirring the mixture overnight and the temperature slowly raised to the room temperature;

(q) filtering and evaporating acetone; and

(r) separating the active ester as a colorless solid.

6. A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, is as follows:

(a) obtaining eggyolk without rupturing the yolk;

(b) adding 100 ml of Tris buffer saline for every 10 ml of yolk;

(c) removing the precipitate by centrifugation;

(d) adding to the supernatant the precipitating solution of magnesium chloride and phosphotungstic acid for centrifuging;

(e) discarding the pellet;

(f) adding to the supernatant a water soluble protein fraction 12% polyethylene glycol;

(g) incubating for 10 minutes and then centrifuging again;

(h) precipitating out the antibody;

(i) adding 10 ml of 10mM phosphate buffer to dissolve the precipitate;

(j) cooling the antibody solution 0^0Cl;

(k) adding 10 ml of pre-cooled ethanol;

(l) centrifuging the solution at 4^0C and dissolving the sediment in 10 mM phosphate buffer; and

(m) dialyzing against phosphate buffer for 24 h at 4^0C to obtain the yield of antibodies.

7. A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, can also be conducted as follows:

(a) obtaining the egg yolk from the eggshell without rupturing the yolk membrane;

(b) adding for every 10 ml of yolk, 10 ml of distilled water;

(c) adding about 0.15 % of kappa- carragenanin and left to stir for 30 minutes at room temperature;

(d) filtering and centrifuging the solution at for 15 minutes;

(e) passing through the DEAE – sephacel column prepared with 20 mM phosphate buffer pH 8.0;

(f) eluting with 0.2 M phosphate buffer pH 8.0;

(g) collecting the eluate and the absorbance read at 280 nm; and

(h) pooling and storing the peak fractions containing the antibody at 4^0 C.

8. A process as claimed in claim 6, wherein the lipid from egg yolk is precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride and centrifuged obtaining the antibody yield up to 75% from supernatant.

9. A process as claimed in claim 6, wherein pH of the water soluble protein fraction obtained after the removal of the lipids is adjusted to pH 5.0 to further precipitate out the antibodies for obtaining a yield of 80 –90%.

10. A process as claimed in claim 7, wherein the yield of antibody is to the extent of 73%.

11. A process as claimed in claim 1, wherein the hyper immune eggs are collected daily and stored 40^0C until further use.

12. A process as claimed in claim 1, wherein commencing the production of the antibody from 7^{th} day after the immunization and continued for 60 days.

13. A process as claimed in claim 1, wherein the titer of the antibody produced is 165-225 mg/ml.

14. A process as claimed in claim 1, wherein production of the egg yolk antibody is more/ equally sensitive to the polyclonal / monoclonal antibodies produced using mammals.

15. A process as claimed in claim 1, wherein production of the egg yolk antibodies relates to small molecules of pesticides.